Ro(SS-A) Positive Sjogren's/Lupus Erythematosus (SC/LE) Overlap Patients Are Associated with the HLA-DR3 and/or DRw6 Phenotypes

Thomas T. Provost, M.D., Norman Talal, M.D., Wilma Bias, Ph.D., John B. Harley, M.D., Ph.D., Morris Reichlin, M.D., and Elaine L. Alexander, M.D., Ph.D.

Departments of Dermatology (TTP) and Medicine (WB, EA), The Johns Hopkins Medical Institutions, Baltimore, Maryland; Division of Clinical Immunology, University of Texas Health Science Center at San Antonio, Texas (NT); Department of Medicine, University of Oklahoma, Arthritis and Immunology Program, Oklahoma Medical Research Foundation, and Veterans Administration Medical Center, Oklahoma City, Oklahoma (JBH); and Immunology Section, University of Oklahoma, and Arthritis and Immunology Lab, Oklahoma Medical Research Foundation, Oklahoma, U.S.A. (MR)

Ro(SS-A) positive female Sjögren's syndrome (SS) lupus erythematosus (LE) overlap patients are a clinically and serologically homogeneous group generally demonstrating prominent subacute cutaneous lupus erythematosus (SCLE) lesions, cutaneous vasculitis, peripheral and central nervous sytem disease, pulmonary disease, and a low frequency of glomerulonephritis. They commonly demonstrate rheumatoid factor, hypergammaglobulinemia, antinuclear and Ro(SS-A) La(SS-B) antibody activity.

This study indicates that these patients are also immunogenetically similar, sharing a statistically significant increased frequency of HLA-B8, DR3, DRW6, DQ2, and DRw52. Sixty-three percent of these SS/LE patients possess the extended haplotype (P-value 6.0×10^{-3}; RR 9.5) HLA-B8, DR3, DQ2, DRw52. One hundred percent of this SS/LE cohort was DR3 or DRw6 (P-value $\leq 5.0 \times 10^{-3}$; relative risk 19.1). Fifty percent of these patients were HLA DR3/DRw6 heterozygotes (P-value 1.5×10^{-6}; relative risk 31.2). Thus, HLA-DR3 and DRw6 Ro(SS-A) positive SS/LE patients may possess a similar, if not unique, DR region DNA nucleotide sequence involved in disease susceptibility or immune regulation. *J Invest Dermatol 91:369–371, 1988*

We recently have described a clinically homogeneous group of female patients with features of Sjögren's syndrome (SS) and lupus erythematosus (LE) (SS/LE) [1]. Clinically, these SS/LE patients display prominent cutaneous lupus erythematosus lesions, most commonly subacute cutaneous lupus erythematosus (SCLE), cutaneous vasculitis, peripheral and central nervous system disease, pulmonary disease, and a low frequency of glomerulonephritis. Serologically, these SS/LE patients demonstrate high titer ($\geq 1.0 \times 10^6$ units/ml) Ro(SS-A) antibodies [60% of whom also have La(SS-B) antibodies], hypergammaglobulinemia, and antinuclear and rheumatoid factor activity.

In order to further investigate the apparent clinical and serologic homogeneity of this group of female SS/LE patients, we performed HLA studies.

MATERIALS AND METHODS

The details of the clinical manifiestations and a description of the cutaneous manifestations of these SS/LE patients have previously been reported [1].

Eight of the original 10 SS/LE patients were HLA typed (one of the original 10 patients was lost to follow-up; the other patient died). HLA typing covered all known A, B, C, DR, and DQ alleles, as well as, DRw52 and DRw53. The standard complement dependent microcytotoxicity assay was used to type the HLA-A, B, and C antigens and the double fluorescent cytotoxicity assay was used for HLA-DR, DQ, DRw52, and DRw53 typing. Controls consisted of 3761 (HLA-A, B, and C) and 1094 (HLA-DR, DQ, DRw52, DRw53) local caucasoids.

Fisher's exact test (two-tailed) was employed for statistical comparisons. Relative risk was determined by the odds ratio method.

RESULTS

The HLA phenotypes of individual patients are presented in Table I. Six patients were HLA-DR3, six were HLA-DRw6, and four were HLA-DR3/DRw6 heterozygotes. All were either HLA-DR3 or DRw6. DQ2 and/or DQ1 was present in all but one patient. All patients had the DRw52 supratypic specificity associated with Sjögren's syndrome.

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Reprint request to: Thomas T. Provost, M.D., Department of Dermatology, Blalock 920, The Johns Hopkins Medical Institutions, 600 North Wolfe Street, Baltimore, MD 21205.

Abbreviations:

HLA: Histocompatible leukocyte antigens.
La(SS-B) antibody: Antibody against a ribonuclear protein.
LE: Lupus erythematosus.
RFLP: Restriction Fragment Length Polymorphism.
Ro(SS-A) antibody: Antibody against a ribonuclear protein.
SCLE: Subacute cutaneous lupus erythematosus.
SS: Sjögren's syndrome.

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TABLE I. HLA-Typing in Sjogren's Syndrome/Lupus Erythematosus (SCLE) Patients.

Patient #	HLA-Loci A	B	C	DR	DO	DRw52
1	11,32	8,—	7,—	2,3	1,2	+
2	1,—	8,60	3,—	3,6	1,2	+
3	2,23	44,45	4	6	1,3	+
4	nT1,36	8,35	3,4	3,6	1,2	+
5	1,—	8,—	—,—	3	2	+
6	36,26	53,71	2,4	3,6	1,2	+
7	3,11	14,22	1,8	1,6	1	+
8	1,3	7,8	—,—	3,6	1,2	+

The frequencies of individual HLA phenotypes in the SS/LE cohort compared to controls are presented in Table II. The SS/LE cohort demonstrates a statistically significant increased frequency of HLA-B8, DR3, DRw6, DQ2, and DRw52. In addition, there was a significant increase in the frequency of HLA-DR3/DRw6 heterozygotes. There was a negative correlation with HLA-DR2. Furthermore, these SS/LE patients had a significant increased frequency of the extended HLA haplotype HLA-B8, DR3, DQ2, DRw52.

The relative risks associated with specific phenotypes were significantly increased (i.e., HLA-B8, DR3, DRw6, DQ1, DQ2, DRw52). The relative risk of the heterozygote HLA-DR3/DRw6 was 31.0. The extended haplotype HLA-B8, DR3, DQ2, DRw52 had a relative risk of 9.5.

DISCUSSION

These studies provide immunogenetic data which support the clinical and serologic data, suggesting homogeneity of this group of female SS/LE patients. These studies also present data indicating the highest relative risks thus far demonstrated between DR alleles and LE or SS.

Previous HLA studies have demonstrated an association of both the HLA-DR2 and DR3 phenotypes with systemic lupus erythematosus [2,3]. Sontheimer et al have demonstrated that there is a statistically significant association of SCLE patients with the HLA-DR3 phenotype and that approximately 70% of SCLE patients are Ro(SS-A) antibody positive [4,5].

Maddison and Bell found that 100% of their Ro(SS-A) positive LE patients were HLA-DR3 positive [6]. Studies from our laboratory have indicated that the Ro(SS-A) antibody in LE is associated with a significant increased frequency of either the HLA-DR2 or DR3 phenotypes [7]. Additional studies in our laboratory suggest that the entire association of HLA-DR2 and DR3 with SLE may be explained by the presence of a Ro(SS-A) antibody response [8]. In a study of 150 SLE patients, we have demonstrated that after the subtraction of the Ro(SS-A) positive LE patients from the total LE cohort, the frequency of the HLA-DR2 and DR3 phenotypes in the remaining non-Ro positive SLE patients was similar to those of normal controls.

Studies by Hochberg et al have demonstrated that the Ro(SS-A) La(SS-B) antibody response in LE is associated with the HLA-DR3 and not the HLA-DR2 phenotype [8]. A statistically significant increased frequency of the La(SS-B) antibody has been detected in the following Ro(SS-A) positive LE subsets: neonatal lupus erythematosus mothers [9,10] and late onset LE (onset of LE after age of 50) [8,11].

Studies of the Ro(SS-A) antibody associations in Sjögren's syndrome have indicated that between 80% and 90% of Ro(SS-A) positive Sjögren's syndrome patients express the HLA-DR2 or DR3 phenotype [12]. Only the Ro-(SS-A) antibody association with the HLA-DR3 phenotype, however, is statistically significant. Subtraction of the Ro(SS-A) antibody positive Sjögren's syndrome patients from the total Sjögren's syndrome patient population removes the statistically significant association of Sjögren's syndrome with the expression of the HLA-DR3 phenotype. Thus, as in lupus erythematosus, the increased frequency of the HLA-DR3 phenotype in SS appears to be associated with the expression of the Ro(SS-A) antibody.

Further studies have indicated that the HLA-DR3 phenotypic expression in the SS patient population is associated with a much higher anti-Ro(SS-A) and anti-La(SS-B) antibody response in comparison to the non-DR3 positive SS patients [13]. Furthermore, the Ro(SS-A) antibody response may be more closely associated with an allele(s) at the HLA-DQ locus. For example, one study indicates that heterozygous DQ1/DQ2 SS patients produce a much greater Ro(SS-A) and La(SS-B) antibody response than those SS patients DQ1 or DQ2 homozygous or heterozygous, but not DQ1/DQ2, at the DQ locus [14]. Similar preliminary studies examining LE patients have also suggested a similar enhanced Ro(SS-A) antibody response associated with DQ1/DQ2 heterozygosity [15].

In addition to the similar increased frequency of the HLA-DR3 phenotype in some LE and SS patients, as well as the association of an enhanced Ro(SS-A) antibody response with the HLA-DQ1/DQ2 alleles, other studies have indicated that the HLA-DRw52 phenotype is also found to be enriched in both SS and some LE patients [7,12]. The HLA-DRw52 antigen is a supratypic specificity (alloantigen) found on the DRβIII chain of haplotypes bearing HLA-DR3, DR4, DRw6, and DR8 alloantigens on the I chain. In one study, 64% of 87 caucasoid SLE patients were HLA-DRw52 positive compared to 40% of 600 caucasoid controls ($P \geq .01$) [7]. At the present time, our initial data suggest that it is the Ro(SS-A) antibody positive late onset SLE patients with SS who frequently have the HLA-DRw52 phenotype. For example, 14 of 16 SS/LE patients (88%) compared to 39 of 74 SLE patients without any accompanying SS (53%) were HLA-DRw52 positive ($P \leq 0.02$) [8].

The present studies also present evidence for an intriguing possi-

Table II. HLA-Typing in Sjogren's Syndrome/Lupus Erythematosus (SCLE) Patients, Comparison with Controls

HLA Phenotype	Frequency (%) Patients	Controls[a]	P-Value	R.R.[b]
B8	63	21	2.4×10^{-2}	6.4
DR2	13	26	0.6	0.4
DR3	75	24	2.0×10^{-3}	19.4
DR6	75	23	61×10^{-3}	9.9
DR3/DRw6	50	3	1.5×10^{-6}	31.2
DR3 or DRw	100	47	5.0×10^{-3}	19.1
DQ1	88	63	0.3	4.1
DQ2	75	42	1.3×10^{-4}	4.2
DQ1/2	63	17	1.0×10^{-4}	8.2
DQ1 or 2	100	100	0.9	7.8×10^{-3}
DRw52	100	63	5.1×10^{-2}	9.9
Extended Haplotype (B8, DR3, DQ2, DRw52)	63	15	6.0×10^{-3}	9.5

[a] Controls #3761 (HLA-A, B and C) and 1094 (HLA-DR, DQ, DRw52, DRw53).
[b] RR: Relative Risk.

ble contribution of HLA-DRw6 to the susceptibility for the development of the SS/LE syndrome. HLA-DR3 appears to have been derived by a gene conversion event involving the DR βI and DR βIII loci of HLA-DRw6 [16]. One hundred percent of this SS/LE patient cohort was either DR3 or DRw6 (P-Value $\leq 5.0 \times 10^{-3}$; relative risk 19.1). Fifty percent of the patients were HLA-DR3/DRw6 heterozygous (P-Value $= 1.5 \times 10^{-}6$; relative risk 31.2). It is conceivable that the HLA-DR3 and DRw6 SS/LE patients may share a similar, if not unique, DR/DQ region DNA nucleotide sequence, such as has been recently demonstrated by the detection of DQ restriction fragment length polymorphisms (RFLPs) in the HLA-DR4, DRw6 Ashkenazic, and non-Ashkenazic Jewish pemphigus vulgaris patients [17].

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